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Association of intraspecific wounding with hantaviral infection in wild rats (*Rattus norvegicus*)

BY G. E. GLASS[1], J. E. CHILDS[1], G. W. KORCH[1] AND J. W. LEDUC[2]

[1] *Department of Immunology and Infectious Diseases, School of Hygiene and Public Health, Johns Hopkins University, 615 N. Wolfe St., Baltimore, MD 21205*

[2] *Department of Epidemiology, U.S. Army Medical Research Institute for Infectious Diseases, Frederick, MD 21701*

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SUMMARY

The potential for hantaviral transmission among wild Norway rats by wounding associated with aggressive interactions was evaluated using a prospective sero-epidemiological study coupled with a mark-release-recapture survey. There was a significant association between an animal's serological status and the presence of wounds. Longitudinal studies of marked and released animals showed seroconversion between captures was associated with wounding between captures more often (33%) than expected by chance, while unwounded animals seroconverted less often (8%) than expected. Typically, less than a 5% difference was found when comparing the incidence of seroconversion with the predicted rate based on wounding and seroprevalence. Infection was highly associated with the onset of sexual maturity and aggression but decoupled from rat age and the length of environmental exposure. Seroconversions occurred at times most associated with aggressive encounters and least associated with amicable behaviours that could lead to aerosol transmission.

INTRODUCTION

Hantaviruses (genus Hantavirus, family *Bunyaviridae*) are the etiological agents of a group of human illnesses collectively referred to as hemorrhagic fever with renal syndrome (HFRS) (Gajdusek, Goldgaber & Millard, 1983). Rodents are their major reservoirs, and disease in humans is associated with close contact with the rodents, their secreta, or excreta. In China and Korea, *Apodemus agrarius* is associated with prototype Hantaan virus (Lee, Lee & Johnson, 1979), while *Clethrionomys glareolus*, and to a lesser extent other microtines, are reservoirs for Puumala virus in northern Eurasia and Scandinavia (Brummer-Korvenkontio *et al.* 1980). *Rattus norvegicus* is a major, worldwide reservoir for Seoul virus and other strains (Lee, Baek & Johnson, 1982; LeDuc *et al.* 1986). Prospect Hill virus primarily infects *Microtus pennsylvanicus* in North America, and currently is the only *Hantavirus* not associated with human disease (Lee *et al.* 1982).

The means by which hantaviruses are maintained and transmitted within

rodent populations are unclear but identifying these processes is significant for understanding the epizootiology and epidemiology of HFRS. Currently, hantaviruses are thought to be transmitted by exposure to fomites and/or aerosols generated from the secreta and excreta of infected animals (LeDuc, 1987). Lee and colleagues (1986) demonstrated transmission of prototype Hantaan virus from experimentally infected *Rattus* to uninfected cage mates after 35 days of exposure. However, the mode of transmission was unclear and the applicability to natural situations was uncertain as prototype Hantaan virus is not normally associated with rats (Lee *et al.* 1986).

Nuzum and colleagues (1988) showed that aerosol exposure to Seoul virus was an effective mode of transmission in outbred Norway rats. However, their study also demonstrated that the infectious dose (ID) of virus varied significantly by the route of exposure, with intramuscular injection at least 200 times more effective than aerosol challenge (ID 50 = 0·003 p.f.u.s *vs.* 0·7 p.f.u.s, respectively). Natural mechanisms that mimic intramuscular or subcutaneous injection, may be a significant factor in hantaviral transmission. Viral transmission by arthropod bites has been attempted on many occasions with generally negative results (Lee *et al.* 1981) but direct intraspecific transmission by bite has been rarely suggested. Hantaviral antigens persist at high titres in the salivary glands and oropharyngeal secretions of infected rats and other rodents (Yanagihara, Amyx & Gajdusek, 1985; Lee *et al.* 1986; H. W. Lee, unpublished data; Yanagihara & Gajdusek, 1987) raising the potential for transmission via saliva during aggressive encounters.

Sexually mature Norway rats engage in intraspecific aggression involving biting so frequently that a large proportion of the adult population exhibits identifiable wounds (Farhang-Azad & Southwick, 1979; Blanchard *et al.* 1985). During a recent study of *Hantavirus* infections in small mammals of Baltimore, Maryland (Childs *et al.* 1987*a*, *b*; Glass *et al.* 1988), data were collected on the incidence and prevalence of hantaviral infection, and size, sex and habitat specific patterns of wounding in Norway rat populations. This paper describes the association between wounding and hantaviral infection, and uses serological status and wounding rates to predict the incidence of infection if transmission is due to infected animals biting uninfected individuals. The results are compared with the observed incidence of seroconversion in four rat populations.

MATERIALS AND METHODS

Sampling procedures

Norway rats were trapped from 1980–6 at 13 sites throughout Baltimore City to provide evidence on the geographic distribution and prevalence of hantaviral infections in rat populations (Childs *et al.* 1985, 1987*b*). Two sites, located in urban parklands, were characterized by grasses, forbs and trees, while 11 sites were located in urban residential areas with high densities of humans. Details of the study areas and trapping protocols are given in Glass *et al.* (1988).

Captured animals were transported to the laboratory and anesthetized, and data recorded on sex, weight, and the presence of wounds. Animals were assigned to one of four 100 g weight classes, with a fifth category for individuals exceeding

400 g. Approximately 0·5 ml of blood was collected by cardiac puncture for serological analysis. Animals from nine sites (eight residential, one parkland) were sacrificed. From September 1984 through June 1986, a mark-release-recapture (MRR) study was undertaken at the four remaining sites to obtain detailed longitudinal information on the dynamics of wounding and hantaviral infection. Trapping protocols were standardized at the MRR sites to equalize the intensity of trapping (Childs *et al.* 1987*b*). Rats in the MRR areas were identified with serially numbered ear tags, and released at their capture sites following recovery from anesthesia. Longitudinal data on serological status, growth rates, and wounding rates were obtained from recaptured animals (Childs *et al.* 1987*b*; Glass, Korch & Childs, 1988; Glass *et al.* 1988).

Wounding studies

Examinations for wounds involved combing each animal's fur, by hand, in all directions so that scars, abscesses, scabs, or areas of hair loss were detected. Wounds were scored into five categories (Glass *et al.* 1988). Unwounded animals were scored as zero. Animals with minor tail wounds were scored as one, while larger wounds on the tail and small wounds ($< 0{\cdot}25$ cm) on the rump, base of the tail or limbs were considered grade 2 wounds. Larger wounds (0·25–0·5 cm) on the body were scored as grade 3 while more extensive wounds were scored as grade 4. Included in the last category were nine rats blinded in one eye and exhibiting heavy scarring around the face. Based on examinations of freshly-made wounds, grades 2–4 were puncture lesions and grades 3 and 4, in particular, involved significant damage to muscle layers.

Differences in wounding between rats from different habitats (residential *vs.* parkland), sexes, and weight classes (main effects) were analysed by multi-way contingency tables. Hierarchical log-linear models were used to test for the significance and independence of main effects (Everitt, 1977).

Changes in wounding status were measured by compiling individual histories of recaptured rats. The number of individuals that remained: unwounded, unchanged, healed, or were wounded, was scored for each time interval between recaptures. An incidence rate of wounding was derived by dividing the number of rats wounded during each time interval by the number of rat-months of observation for that interval. Size-specific wounding rates were estimated for each of the five weight classes, using the average weight between recaptures.

Serological methods and measures of incidence

Sera were separated and stored at -20 °C until tested for antibodies with Vero E-6 cells infected with Hantaan virus (strain 76-118), using spot slides (prepared by Dr G. French, Salk Institute; Swiftwater, PA). The indirect fluorescent antibody (IFA) test is described in detail by LeDuc, Smith & Johnson (1984). Sera were screened at 1 in 8 and 1 in 32 dilutions using FITC-conjugated goat anti-rat IgG (heavy and light chain specific; Cappel Laboratories; Westchester, PA). Sera with titres $\geqslant 32$ were considered positive and were titrated to endpoints in fourfold dilutions. Prevalence rates were determined separately, for each sex, from each habitat, in each of the weight classes.

To reduce the potential confounding effects of maternal antibody or nonspecific

reactions on seroprevalence and incidence, we used three IFA titre classes to examine the associations between the degree of wounding and serological status; seronegative (IFA < 32), low seropositive ($32 \leqslant$ IFA < 128) and high seropositive (IFA $\geqslant 128$). These categories correlate with the presence of neutralizing antibodies that are found almost exclusively in the high seropositive class (Childs *et al.* 1985; Tsai *et al.* 1985). We also combined wound grades 3 and 4 into a single class for further analysis because they were distinguished primarily by the size of the wounds.

Incidence rates of infection among marked and released rats were estimated by procedures described by Childs *et al.* (1987*b*). Animals showing a $\geqslant$ fourfold rise in titre were considered to have become infected. The number of these animals was used as the numerator for determining the incidence rate of infection in each MRR population. The populations at risk (denominators) were the number of seronegative rats alive in each population. The number of animal-months of exposure for the at-risk population was determined by summing the time intervals during which the animals remained seronegative and adding half the interval between recaptures for animals whose serological status changed between captures (Childs *et al.* 1987*b*).

The predicted incidence of seroconversion due to biting at each MRR site was calculated by multiplying the incidence rate for wounding by the proportion of seropositive animals among rats $\geqslant 200$ g at the site. Smaller rats were excluded from the calculation as they were rarely wounded (Glass *et al.* 1988), rarely seropositive (Childs *et al.* 1985), and previous studies showed that aggressor rats were large, sexually mature animals (Calhoun, 1962).

Ageing techniques

Ages at seroconversion were estimated using published growth rates of *R. norvegicus* from these Baltimore populations (Glass, Korch & Childs, 1988), and Slade, Sauer & Glass' (1984) method for growth rate analysis of free-ranging small mammals. Changes in body weight between capture intervals were divided by the time intervals between captures to estimate growth rates (g/month). Composite growth curves were constructed for animals in each habitat by taking a hypothetical 20 g weanling rat (Calhoun, 1962; Frazer, 1977) and applying the appropriate seasonal, weight-specific growth rate for the habitat. This value was projected to the following time interval and the process iterated to describe the average growth of individuals.

The growth rate data were used to estimate the age of seroconverting rats by taking the weight at the time mid-way between captures and backcalculating to find the most recent birthmonth that would produce a rat with the observed body weight. This estimate was accurate as most seroconversions occurred during intervals of less than 2 months (Childs *et al.* 1987*b*).

RESULTS

Sampling

Rats were captured most frequently in residential areas ($n = 542$) and were predominantly males (Table 1), particularly at the parkland sites where the excess

Table 1. *Numbers of Norway rats in five mass classes from residential and parkland sites. Totals exclude 55 captures made within the same sampling interval*

	Mass class (g)					
	< 100	100–199	200–299	300–399	> 400	Total
Residential						
Male	27	58	34	71	94	284
Female	32	69	45	48	64	258
Total	59	127	79	119	158	542
Parkland						
Male	6	29	37	18	3	93
Female	9	22	20	13	0	64
Total	15	51	57	31	3	157
Total	74	178	136	150	161	699

Table 2. *Frequency of wounding in residential and parkland Norway rats. Grades are defined in the text*

	Wounding grade					
	0	1	2	3	4	Total
Residential						
Male	122	56	29	24	26	257
Female	128	32	33	25	4	222
Total	250	88	62	49	30	479
Parkland						
Male	57	5	11	2	1	76
Female	48	2	3	0	0	53
Total	105	7	14	2	1	129
Total	355	95	76	51	31	608

was significant ($\chi^2 = 4.57$; $0{\cdot}025 < P < 0{\cdot}05$). Residential rats, of both sexes, were significantly larger than their parkland counterparts (ANOVA $F = 34{\cdot}17$, D.F. = 1, 751; $P < 0{\cdot}0001$). This was due to the large proportion of residential rats weighing $\geqslant 400$ g (29·2%; $n = 158$). Only three (1·9%) parkland rats exceeded this weight. Males also were significantly larger than females in both habitats ($F = 7{\cdot}17$; D.F. = 1, 751; $P < 0{\cdot}01$).

We tagged and released 372 rats as part of the MRR protocol and 107 (28·8%) were recaptured 146 times. Ninety-one captures occurred over intervals of at least 1 month (range 1–10 months) while 55 occurred within the same 3-day trapping period.

Wounding

Wounding and serological records were obtained from 608 rats captured 754 times. A total of 253 (41·6%) rats had wounds when captured (Table 2). Most wounded rats had tail wounds (grade 1; 37·5%) or minor wounds on their rumps and limbs (grade 2; 30·0%), but approximately a third (32·5%) had serious (grades 3 or 4) wounds. Wounding increased with weight in both sexes, although the weight-specific pattern differed between the sexes. Wounding was first noted

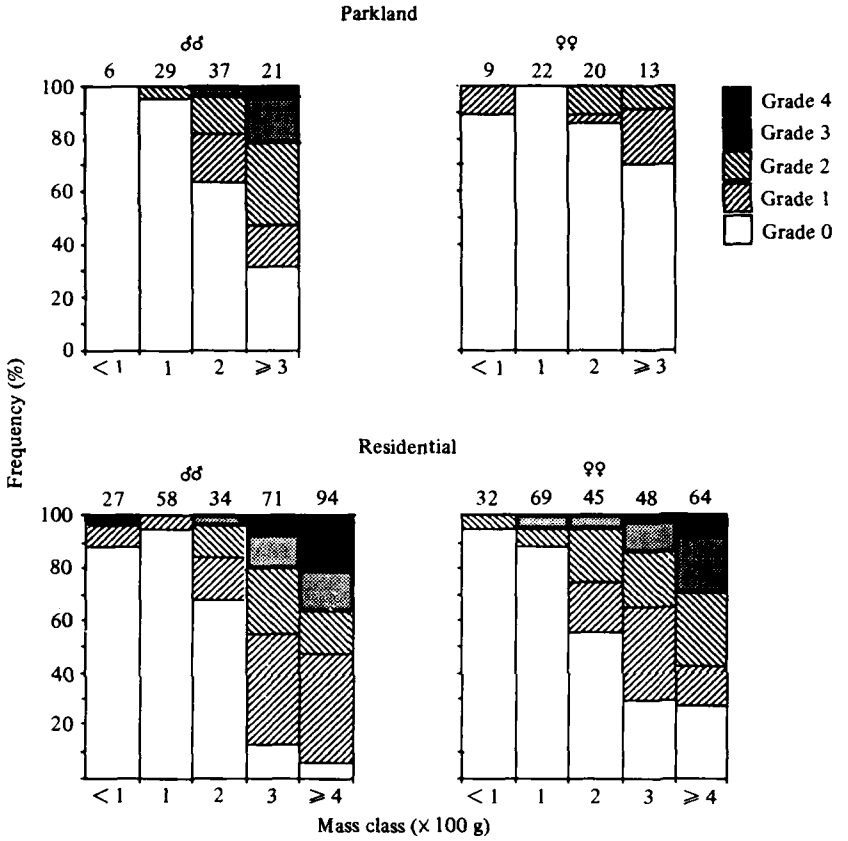

Fig. 1. Frequencies of wounding for parkland and residential males and females by weight classes. Wounding grades (0 = none to 4 = severe) are defined in the text (Materials and Methods).

when animals of either sex reached approximately 200 g, but wounding was less severe among females within any weight class (Fig. 1). In parklands, the overall wounding rate was lower, for both sexes, than in residential locales (Fig. 1): 54·7% of residential males and 42·9% of residential females were wounded compared with 29·8% and 12·5% of parkland males and females, respectively.

A significant reduction in the variation of wounding scores was obtained using any one of the three main effects (weight, sex, or habitat) in the log-linear model. A model using a linear combination of body weight and sex gave the greatest reduction in the residual variation indicating the significant of these effects on wounding (χ^2 weight = 142·16; D.F. = 9; $P < 0{\cdot}0001$; χ^2 sex = 11·83; D.F. = 4; $P = 0{\cdot}02$). The interaction between these terms was not statistically significant ($\chi^2 = 11{\cdot}0$; D.F. = 9; $P = 0{\cdot}27$), implying that body weight and sex acted independently on wounding. Including the habitat variable did not significantly reduce the residual variation explained by the weight and sex model ($\chi^2 = 4{\cdot}23$; D.F. = 4; $P = 0{\cdot}38$). Therefore, differences in habitat were considered not significant in predicting wounding patterns. The differences in wounding between parkland and residential rats appears entirely due to the absence of large ($\geqslant 400$

g) rats in parklands as wounding prevalences were very similar within weight classes between the two habitats (Fig. 1). Similarly, the major difference in wounding between sexes, within any mass class, was due to the greater severity of wounds among males, rather than in the prevalence of wounding (Fig. 1).

Thirty-one (34·1%) rats were wounded between captures occurring over at least 1 months' interval (median time between captures = 2·5 months; range 1–10). In 39·6% ($n = 36$) of the recaptures, rats were not wounded (median time = 2 months, range 1–5). For 26·4% of recaptures, rats were initially wounded and either showed marked healing (15·4%) or remained unchanged (11·0%). In both cases the median resampling interval was 1 month (ranges 1–4 and 1–8 months, respectively). Only one rat was wounded during the 3-day interval within a trapping period ($n = 55$).

Wounds healed rapidly in the absence of secondary infection. Of 14 rats whose wounds improved, 4 with grade 1 or 2 wounds were completely healed within 2 months. When wounds were severe (grades 3 or 4; $n = 6$) scabs were present for up to 4 months. In three cases wounds became secondarily infected and persisted as open, inflamed sores for up to 8 months.

Based on all 91 recaptures, 222 rat-months of observations were obtained, giving an incidence rate for wounding of 14·0%/month of observation ($n = 31$). Considering only animals with recapture intervals of 2 months or less more accurately reflects wounding rates by eliminating animals whose wounds may have healed. During these 91 months of observations 15 rats were wounded and the incidence was estimated as *16·5%/month*.

Wounding did not occur equally among all weight classes. No rats less than 100 g were wounded between recaptures. Wounding increased to 8%/month ($n =$ 25 rat-months) in the 100–199 g class and peaked at 20·3%/month in the 200–299 g class ($n = 69$ rat-months). The rate of wounding decreased slightly in the larger classes (17·0 and 17·8%/month; 47 and 45 rat-months, respectively). Overall, the incidence of wounding in rats $\geqslant$ 200 g was 18·6%/month.

Serology

The overall seroprevalence to a hantavirus, based on 703 blood samples, was 38·9% but the proportions of seropositive animals varied with body size (Fig. 2). There were no differences between sexes. The proportions of seropositive animals in weight classes initially decreased until 200–299 g and then increased in larger rats. Evidence suggests that antibody in small rats (< 200 g) is usually maternal in origin and does not reflect acute infection (Childs *et al.* 1987*b*). This is supported by the observation that 84 of 87 (96·6%) of the seropositive small (< 200 g) rats had antibody titres $\leqslant$ 128 and 5 of 7 (71·4%) had lower titres (four became seronegative) at recapture. In contrast, 82·4% of $\geqslant$ 200 g seropositive rats had titres $\geqslant$ 128 and only 1 of 61 (1·6%) decreased its titre between captures.

There were highly significant associations between the presence of wounds and an animal's serological status, for both sexes ($\chi^2 = 37{\cdot}95$; 6 D.F.; $P < 0{\cdot}0001$ males; $\chi^2 = 18{\cdot}80$; 6 D.F.; $P = 0{\cdot}02$ females). The majority of the effect was due to the excess of animals with high titres that had severe body wounds ($\chi^2 = 12{\cdot}6$ males; $\chi^2 = 3{\cdot}4$ females) and a paucity of high titred animals without body

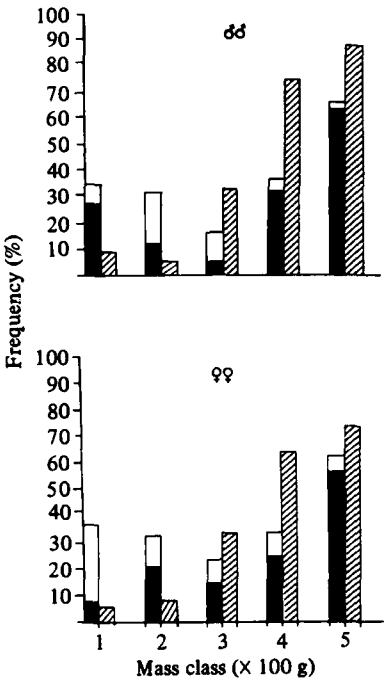

Fig. 2. Frequencies of wounding and seropositivity (low seropositive open bars; high seropositive closed bars) for male and female rats by weight classes. ■, Seropositive > 1:128; □, seropositive > 1:32; ▨, wounded.

Table 3. *Serological status and wounding history between captures for marked and released Norway rats. Six rats with unknown serological histories are not included*

	Serological status			
Wounding history	Negative	Positive at initial capture	Seroconverted	Total
Not wounded	23	7	2	32
Wounded at initial capture	10	10	10	30
Wounded between captures	14	2	7	23
Total	47	19	19	85

wounds ($\chi^2 = 11{\cdot}0$ males; $\chi^2 = 3{\cdot}2$ females). The next largest contribution was due to the lack of seronegative animals with body wounds.

Of the 85 rats recaptured and rebled, 19 seroconverted. Most (61·2 %) went from seronegative to titres exceeding 2048. Among recaptured animals there was a statistically significant association between their serological status and wounding history ($\chi^2 = 13{\cdot}9$; 4 D.F.; $P < 0{\cdot}01$; Table 3). Animals that were not wounded between recaptures seroconverted less often than expected if wounding and serological status were independent ($\chi^2 = 5{\cdot}3$). Only two animals that were not wounded between captures seroconverted. One, recaptured after 4 months, had seroconverted to high titre but the time interval was sufficient for wounds to heal (see above). The other was recaptured after 2 months and had gone from

Table 4. *The predicted and observed seroconversion rates among Norway rats at four MRR study sites. Months of observation is the denominator for the incidence function of wounding*

MRR site	Months of observation	Wounding incidence (% month)	Sero-prevalence	Predicted seroconversion (% month)	Observed seroconversion (% month)
Winston Govane	60·5	21·5	0·48	10·2	14·6
Washington	41·0	19·5	0·35	6·9	7·0
Chase	18·5	28·2	0·51	14·5	46·2
Cherry Hill	36·0	16·7	0·45	7·5	9·2
Total	156·0	20·7	—	9·4	12·1

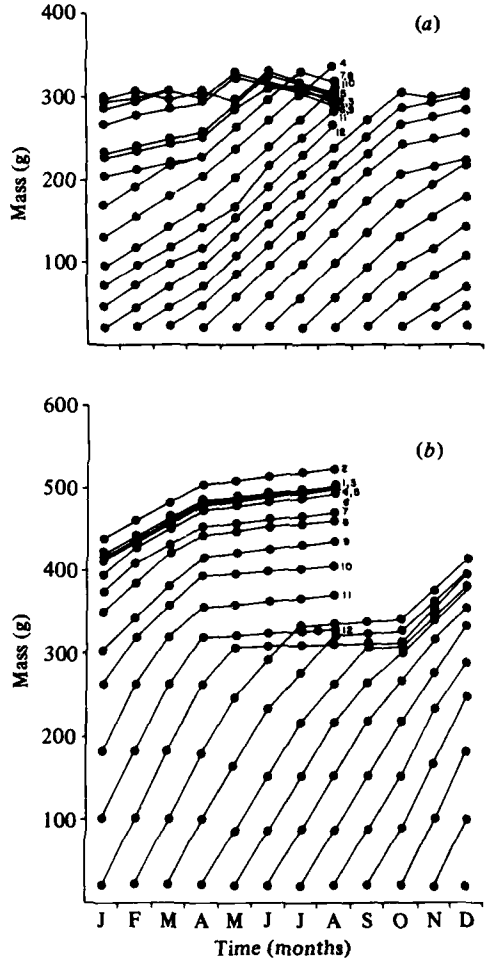

Fig. 3. Hypothetical growth curves for parkland (*a*) and residential (*b*) rats during each month of the year.

seronegative to an IFA titre of 64. Animals that were wounded when first caught, or became wounded between recaptures seroconverted more often than expected due to chance ($\chi^2 = 2{\cdot}3$). Animals wounded between captures were also less likely to be seropositive at first capture than expected ($\chi^2 = 1{\cdot}9$).

The predicted infection rates for rats $\geqslant 200$ g at each of the MRR sites ranged from 6·9 to 14·5%/month (Table 4). The predicted infection rate among parkland rats (7·5%/month) was within the range of values predicted for rats in residential locations. Although the incidence of wounding in parkland rats was lower than for rats from residential sites, the higher seroprevalence among $\geqslant 200$ g parkland rats counterbalanced the decreased wounding. Among residential populations, two sites (Winston Govane and Washington) had nearly equal wounding incidence rates, but the predicted seroconversion rate was 48% higher at one site due to the higher seroprevalence among larger rats at that site. The highest predicted seroconversion rate occurred at the third residential location (Chase) where both the seroprevalence and wounding incidence were maximal (Table 4).

Overall, the predicted infection rate (9·4%/month) closely agreed with the observed infection rate (12·1%/month). The predicted infection rates paralleled the observed rates, differing between 0·1%/month and 4·4%/month at three of the four MRR sites (Table 4). At the fourth site, the small sample size (6·5 rat-months) may account for the extreme value of the observation. Recalculating the overall infection rate excluding this site gave predicted and observed rates of 8·6%/month and 10·4%/month, respectively.

Growth rates and age estimations

The composite growth curves used for age estimates of seroconverting rats are shown in Fig. 3. Parkland rats grew more slowly than rats from residential areas and ceased growing at 300–400 g, while residential rats continued to grow. As a result, rats from residential sites tended to be younger than parkland rats of comparable sizes. Seroconverting rats in residential areas were significantly younger than rats from parklands ($U = 55$, $P < 0{\cdot}05$). The median age at seroconversion was 6 months (range 3 to 13 months) among residential rats and 11 months (8–14 months) among parkland rats.

DISCUSSION

The natural mode(s) of hantaviral transmission within reservoir species remains unclear and the role of intraspecific aggression remains necessarily speculative. Aerosol transmission is thought to be the major route of infection to humans, with most people exposed after handling infected rodents or close contact with their nest sites, burrows, or bedding (Desmyter *et al.* 1983; Dournon *et al.* 1984; LeDuc, 1987; Yanagihara & Gajdusek, 1987). However, the mechanisms of intraspecific transmission may differ from interspecific routes and it is critical to identify these modes to understand the dynamics of infection within a reservoir population. For example, hantaviral transmission could spread more rapidly through a population by biting, involving unrelated animals fighting at territorial boundaries, than by aerosols requiring close social contact. Similarly, hantaviruses may be more uniformly distributed through a population by biting, while microfoci of infections

(e.g. within burrow systems, and family units) might be expected if transmission occurs by means requiring social contact.

The close agreement between the observed and expected rates of infection, assuming transmission by random aggressive encounters between infected and uninfected mature rats, suggests intraspecific aggression may play an important role in hantaviral transmission among rats. In particular, there is a perfect rank correlation between the predicted and observed incidence rates of infection resulting from wounding and seroconversion in each population. Several other lines of evidence also support our conclusions.

First, antigen is known to persist in salivary glands and saliva for extended periods of time following infection and is associated with periods of infectiousness in several rodent reservoirs (Lee *et al.* 1981; Yanagihara, Amyx & Gajdusek, 1985; Lee *et al.* 1986; Yanagihara & Gajdusek, 1987). There is at least one reported case of HFRS in humans following a bite by a wild rodent, implicating this mechanism in the transmission of hantaviruses to humans (Dournon *et al.* 1984). A case of HFRS due to a bite from an infected laboratory rat also is suspected (Professor T. Yamanouchi; Osaka University, personal communication), and trench nephritis, similar to nephropathia epidemica during World War I has been associated with rodent bite (Boulangier & Martin, 1917, cited in Clemment, 1987). Rodent to human transmission by this route may be limited more by the relative rarity of rodents biting people than other factors. Secondly, the extremely low doses (0·003 p.f.u.s) of rat-associated hantaviruses necessary for transmission by intramuscular routes are over two orders of magnitude lower than those required for successful aerosol challenge (Nuzum *et al.* 1988). Wounding grades 2–4 are often puncture lesions that involve penetration of muscle and may be analogous to intramuscular inoculation.

Finally, the epidemiological patterns of infection among rodents in this study indicate that wounding and infection patterns are highly associated with one another, but are inconsistent with the hypothesis of transmission by aerosols during times of close, amicable interactions. While the overall correlation between wounding and serological status with increasing body weight is consistent with transmission by biting, it could also occur if wounding and infection were simply independent functions of time and not causally related.

Three observations implicate aggressive encounters in transmission of this virus. First, the wounding of individuals was significantly associated with their seroconversion. Only two individuals (8%) that showed no evidence of wounding between recaptures seroconverted. In contrast, 33% ($n = 21$) of the seronegative rats bitten between recaptures seroconverted. Despite the difference in seroconversion rates, the time wounded and unwounded animals remained in the environment before resampling did not differ significantly (2·5 and 2·0 months, respectively). Thus, time in the environment and the potential for exposure to virus appears insufficient to account for the observed changes in serological status.

Second, seroconversions were size-related rather than age-related. Rats in Baltimore seroconverted at an average body weight of 306 g (Childs *et al.* 1987*b*), but rats in parklands were nearly twice as old (11 months) as residential rats (6 months) when they seroconverted. Wounding followed a similar weight-specific

rather than age-specific pattern, as indicated by the presence of size effects and the absence of habitat effects in the log-linear model. Extensive aggressive interactions begin only after juveniles reach sexual maturity (Calhoun, 1962), and in Baltimore this occurs after rats reach 200 g. This body size threshold is independent of age among parkland and residential rats of both sexes (Glass *et al.* 1988), and may be controlled by a nutritional threshold required for breeding, as has been demonstrated among laboratory rats (Bronson, 1987). Thus, parkland rats apparently do not engage in aggressive encounters until they are much older than residential rats (6–7 months versus 2 months, respectively), and similarly seroconversion is delayed. This would account for the overall differences in both wounding and seroprevalence (Childs *et al.* 1987*a*) between parkland and residential populations. Habitat differences in the age of seroconversion would not be expected if aerosol transmission predominated, unless perinatal social behaviour differed markedly in residential and parkland habitats. Juvenile rats should be exposed in their natal burrows where there is extensive grooming and social contact (Calhoun, 1962; Hart, 1982).

Third, the seasonal pattern of transmission does not indicate a single peak in seroconversions (Childs *et al.* 1987*b*). Arikawa *et al.* (1986) proposed that transmission occurred in burrows during the winter when rats may huddle together and be in close contact. If true, transmission and seroconversions would peak primarily during late winter months. Their data indicated it was primarily overwintering older rats that were infected, while overwintering younger rats were rarely seropositive. This discrepancy was explained by the possibly inefficient transmission of hantaviruses. However, Childs *et al.* (1987*b*) noted that seroconversions occurred throughout the year and most occurred during the spring and autumn; times when wounding peaked with reproductive activity (Glass *et al.* 1988). In addition, inefficient transmission does not account for the rarity of young, seropositive, rats as our data show hantaviral transmission is very efficient in natural populations, approaching rates seen for some viral infections among closed colonies of laboratory rats (Robinson, Nathanson & Hodous, 1971).

In summary, our data indicate that there is good evidence hantaviral infections may be maintained, at least in part, within rat populations through biting during aggressive interactions. The evidence is threefold: there is a strong association throughout the populations studied between the presence of wounds and infection; among individuals followed prospectively there is a close temporal association between wound acquisition and seroconversion and; finally, infections appear decoupled from age, but highly associated with the onset of reproduction and aggressive behaviour. The wounding incidence and seroprevalence rate accurately predict the incidence of seroconversions in the populations. While laboratory studies (e.g. Nuzum *et al.* 1988) indicate feasible modes of transmission, they do not identify the predominant route in natural populations. Clarifying transmission dynamics of infection may depend on comparing how well the alternative exposure routes can predict patterns of infections in free-ranging populations. The relative importance we attach to each route in the transmission of hantaviruses will influence our views of the epidemiology and control of infection and need to be evaluated.